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SEQUENCE LISTING

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CHEN, JUI-LIN  
YANG, NING-SUN

<120> A TRUNCATED FORM OF FIBROBACTER SUCCINOGENES 1, 3-1,  
4-BETA-D-GLUCANASE WITH IMPROVED ENZYMATIC ACTIVITY AND  
THERMO-TOLERANCE

<130> 4910-8

<140> 09/654,652

<141> 2000-09-05

<160> 11

<170> PatentIn Ver. 2.1

<210> 1

<211> 248

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Modified enzyme  
with enhanced activity and thermal stability

<400> 1

Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu  
1 5 10 15

Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala  
20 25 30

Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile  
35 40 45

Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys  
50 55 60

Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala  
65 70 75 80

Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln  
85 90 95

Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp  
100 105 110

Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser  
115 120 125

Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu  
130 135 140

Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe  
145 150 155 160

Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly  
 165 170 175

Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr  
 180 185 190

Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn  
 195 200 205

Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu  
 210 215 220

Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val  
 225 230 235 240

Pro Arg Asp Asp Glu Pro Ala Pro  
 245

<210> 2

<211> 267

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Modified enzyme  
 with enhanced activity and thermal stability

<400> 2

Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu  
 1 5 10 15

Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala  
 20 25 30

Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile  
 35 40 45

Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys  
 50 55 60

Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala  
 65 70 75 80

Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln  
 85 90 95

Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp  
 100 105 110

Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser  
 115 120 125

Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu  
 130 135 140

Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe  
 145 150 155 160  
 Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly  
 165 170 175  
 Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr  
 180 185 190  
 Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn  
 195 200 205  
 Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu  
 210 215 220  
 Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val  
 225 230 235 240  
 Pro Arg Asp Asp Glu Pro Ala Pro Asn Ser Ser Ser Val Asp Lys Leu  
 245 250 255  
 Ala Ala Ala Leu Glu His His His His His  
 260 265

<210> 3  
 <211> 349  
 <212> PRT  
 <213> Fibrobacter succinogenes

<400> 3  
 Met Asn Ile Lys Lys Thr Ala Val Lys Ser Ala Leu Ala Val Ala Ala  
 1 5 10 15  
 Ala Ala Ala Ala Leu Thr Thr Asn Val Ser Ala Lys Asp Phe Ser Gly  
 20 25 30  
 Ala Glu Leu Tyr Thr Leu Glu Glu Val Gln Tyr Gly Lys Phe Glu Ala  
 35 40 45  
 Arg Met Lys Met Ala Ala Ala Ser Gly Thr Val Ser Ser Met Phe Leu  
 50 55 60  
 Tyr Gln Asn Gly Ser Glu Ile Ala Asp Gly Arg Pro Trp Val Glu Val  
 65 70 75 80  
 Asp Ile Glu Val Leu Gly Lys Asn Pro Gly Ser Phe Gln Ser Asn Ile  
 85 90 95  
 Ile Thr Gly Lys Ala Gly Ala Gln Lys Thr Ser Glu Lys His His Ala  
 100 105 110  
 Val Ser Pro Ala Ala Asp Gln Ala Phe His Thr Tyr Gly Leu Glu Trp  
 115 120 125  
 Thr Pro Asn Tyr Val Arg Trp Thr Val Asp Gly Gln Glu Val Arg Lys  
 130 135 140

Thr Glu Gly Gly Gln Val Ser Asn Leu Thr Gly Thr Gln Gly Leu Arg  
 145 150 155 160  
 Phe Asn Leu Trp Ser Ser Glu Ser Ala Ala Trp Val Gly Gln Phe Asp  
 165 170 175  
 Glu Ser Lys Leu Pro Leu Phe Gln Phe Ile Asn Trp Val Lys Val Tyr  
 180 185 190  
 Lys Tyr Thr Pro Gly Gln Gly Glu Gly Ser Asp Phe Thr Leu Asp  
 195 200 205  
 Trp Thr Asp Asn Phe Asp Thr Phe Asp Gly Ser Arg Trp Gly Lys Gly  
 210 215 220  
 Asp Trp Thr Phe Asp Gly Asn Arg Val Asp Leu Thr Asp Lys Asn Ile  
 225 230 235 240  
 Tyr Ser Arg Asp Gly Met Leu Ile Leu Ala Leu Thr Arg Lys Gly Gln  
 245 250 255  
 Glu Ser Phe Asn Gly Gln Val Pro Arg Asp Asp Glu Pro Ala Pro Gln  
 260 265 270  
 Ser Ser Ser Ala Pro Ala Ser Ser Ser Val Pro Ala Ser Ser  
 275 280 285  
 Ser Ser Val Pro Ala Ser Ser Ser Ala Phe Val Pro Pro Ser Ser  
 290 295 300  
 Ser Ser Ala Thr Asn Ala Ile His Gly Met Arg Thr Thr Pro Ala Val  
 305 310 315 320  
 Ala Lys Glu His Arg Asn Leu Val Asn Ala Lys Gly Ala Lys Val Asn  
 325 330 335  
 Pro Asn Gly His Lys Arg Tyr Arg Val Asn Phe Glu His  
 340 345

<210> 4  
 <211> 744  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: DNA encoding a  
 modified enzyme

<220>  
 <221> CDS  
 <222> (1)..(744)

<400> 4  
 atg gtt agc gca aag gat ttt agc ggt gcc gaa ctc tac acg tta gaa  
 Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu  
 1 5 10 15

gaa gtt cag tac ggt aag ttt gaa gcc cgt atg aag atg gca gcc gca Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala	20                    25                    30	96
tcg gga aca gtc agt tcc atg ttc ctc tac cag aat ggt tcc gaa atc Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile	35                    40                    45	144
gcc gat gga agg ccc tgg gta gaa gtg gat att gaa gtt ctc ggc aag Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys	50                    55                    60	192
aat ccg ggc agt ttc cag tcc aac atc att acc ggt aag gcc ggc gca Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala	65                    70                    75                    80	240
caa aag act agc gaa aag cac cat gct gtt agc ccc gcc gcc gat cag Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln	85                    90                    95	288
gct ttc cac acc tac ggt ctc gaa tgg act ccg aat tac gtc cgc tgg Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp	100                    105                    110	336
act gtt gac ggt cag gaa gtc cgc aag acg gaa ggt ggc cag gtt tcc Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser	115                    120                    125	384
aac ttg aca ggt aca cag gga ctc cgt ttt aac ctt tgg tcg tct gag Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu	130                    135                    140	432
agt gcg gct tgg gtt ggc cag ttc gat gaa tca aag ctt ccg ctt ttc Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe	145                    150                    155                    160	480
cag ttc atc aac tgg gtc aag gtt tat aag tat acg ccg ggc cag ggc Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly	165                    170                    175	528
gaa ggc ggc agc gac ttt acg ctt gac tgg acc gac aat ttt gac acg Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr	180                    185                    190	576
ttt gat ggc tcc cgc tgg ggc aag ggt gac tgg aca ttt gac ggt aac Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn	195                    200                    205	624
cgt gtc gac ctc acc gac aag aac atc tac tcc aga gat ggc atg ttg Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu	210                    215                    220	672
atc ctc gcc ctc acc cgc aaa ggt cag gaa agc ttc aac ggc cag gtt Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val	225                    230                    235                    240	720

ccg aga gat gac gaa cct gct ccg Pro Arg Asp Asp Glu Pro Ala Pro 245	744
<210> 5	
<211> 804	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Description of Artificial Sequence: DNA encoding a modified enzyme	
<220>	
<221> CDS	
<222> (1)..(804)	
<400> 5	
atg gtt agc gca aag gat ttt agc ggt gcc gaa ctc tac acg tta gaa Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu	48
1 5 10 15	
gaa gtt cag tac ggt aag ttt gaa gcc cgt atg aag atg gca gcc gca Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala	96
20 25 30	
tcg gga aca gtc agt tcc atg ttc ctc tac cag aat ggt tcc gaa atc Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile	144
35 40 45	
gcc gat gga agg ccc tgg gta gaa gtg gat att gaa gtt ctc ggc aag Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys	192
50 55 60	
aat ccg ggc agt ttc cag tcc aac atc att acc ggt aag gcc ggc gca Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala	240
65 70 75 80	
caa aag act agc gaa aag cac cat gct gtt agc ccc gcc gcc gat cag Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln	288
85 90 95	
gct ttc cac acc tac ggt ctc gaa tgg act ccg aat tac gtc cgc tgg Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp	336
100 105 110	
act gtt gac ggt cag gaa gtc cgc aag acg gaa ggt ggc cag gtt tcc Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser	384
115 120 125	
aac ttg aca ggt aca cag gga ctc cgt ttt aac ctt tgg tcg tct gag Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu	432
130 135 140	
agt gcg gct tgg gtt ggc cag ttc gat gaa tca aag ctt ccg ctt ttc Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe	480
145 150 155 160	

cag ttc atc aac tgg gtc aag gtt tat aag tat acg ccg ggc cag ggc Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly 165 170 175	528
gaa ggc ggc agc gac ttt acg ctt gac tgg acc gac aat ttt gac acg Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr 180 185 190	576
ttt gat ggc tcc cgc tgg ggc aag ggt gac tgg aca ttt gac ggt aac Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn 195 200 205	624
cgt gtc gac ctc acc gac aag aac atc tac tcc aga gat ggc atg ttg Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu 210 215 220	672
atc ctc gcc ctc acc cgc aaa ggt cag gaa agc ttc aac ggc cag gtt Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val 225 230 235 240	720
ccg aga gat gac gaa cct gct ccg aat tcg agc tcc gtc gac aag ctt Pro Arg Asp Asp Glu Pro Ala Pro Asn Ser Ser Val Asp Lys Leu 245 250 255	768
gcg gcc gca ctc gag cac cac cac cac cac tga Ala Ala Ala Leu Glu His His His His His His 260 265	804
<210> 6	
<211> 1050	
<212> DNA	
<213> Fibrobacter succinogenes	
<220>	
<221> CDS	
<222> (1)..(1047)	
<400> 6	
atg aac atc aag aaa act gca gtc aag agc gct ctc gcc gta gca gcc Met Asn Ile Lys Lys Thr Ala Val Lys Ser Ala Leu Ala Val Ala Ala 1 5 10 15	48
gca gca gca gcc ctc acc acc aat gtt agc gca aag gat ttt agc ggt Ala Ala Ala Leu Thr Thr Asn Val Ser Ala Lys Asp Phe Ser Gly 20 25 30	96
gcc gaa ctc tac acg tta gaa gaa gtt cag tac ggt aag ttt gaa gcc Ala Glu Leu Tyr Thr Leu Glu Glu Val Gln Tyr Gly Lys Phe Glu Ala 35 40 45	144
cgt atg aag atg gca gcc gca tcg gga aca gtc agt tcc atg ttc ctc Arg Met Lys Met Ala Ala Ala Ser Gly Thr Val Ser Ser Met Phe Leu 50 55 60	192

tac cag aat ggt tcc gaa atc gcc gat gga agg ccc tgg gta gaa gtg Tyr Gln Asn Gly Ser Glu Ile Ala Asp Gly Arg Pro Trp Val Glu Val 65 70 75 80	240
gat att gaa gtt ctc ggc aag aat ccg ggc agt ttc cag tcc aac atc Asp Ile Glu Val Leu Gly Lys Asn Pro Gly Ser Phe Gln Ser Asn Ile 85 90 95	288
att acc ggt aag gcc ggc gca caa aag act agc gaa aag cac cat gct Ile Thr Gly Lys Ala Gly Ala Gln Lys Thr Ser Glu Lys His His Ala 100 105 110	336
gtt agc ccc gcc gcc gat cag gct ttc cac acc tac ggt ctc gaa tgg Val Ser Pro Ala Ala Asp Gln Ala Phe His Thr Tyr Gly Leu Glu Trp 115 120 125	384
act ccg aat tac gtc cgc tgg act gtt gac ggt cag gaa gtc cgc aag Thr Pro Asn Tyr Val Arg Trp Thr Val Asp Gly Gln Glu Val Arg Lys 130 135 140	432
acg gaa ggt ggc cag gtt tcc aac ttg aca ggt aca cag gga ctc cgt Thr Glu Gly Gly Gln Val Ser Asn Leu Thr Gly Thr Gln Gly Leu Arg 145 150 155 160	480
ttt aac ctt tgg tcg tct gag agt gcg gct tgg gtt ggc cag ttc gat Phe Asn Leu Trp Ser Ser Glu Ser Ala Ala Trp Val Gly Gln Phe Asp 165 170 175	528
gaa tca aag ctt ccg ctt ttc cag ttc atc aac tgg gtc aag gtt tat Glu Ser Lys Leu Pro Leu Phe Gln Phe Ile Asn Trp Val Lys Val Tyr 180 185 190	576
aag tat acg ccg ggc cag ggc gaa ggc ggc agc gac ttt acg ctt gac Lys Tyr Thr Pro Gly Gln Gly Glu Gly Ser Asp Phe Thr Leu Asp 195 200 205	624
tgg acc gac aat ttt gac acg ttt gat ggc tcc cgc tgg ggc aag ggt Trp Thr Asp Asn Phe Asp Thr Phe Asp Gly Ser Arg Trp Gly Lys Gly 210 215 220	672
gac tgg aca ttt gac ggt aac cgt gtc gac ctc acc gac aag aac atc Asp Trp Thr Phe Asp Gly Asn Arg Val Asp Leu Thr Asp Lys Asn Ile 225 230 235 240	720
tac tcc aga gat ggc atg ttg atc ctc gcc ctc acc cgc aaa ggt cag Tyr Ser Arg Asp Gly Met Leu Ile Leu Ala Leu Thr Arg Lys Gly Gln 245 250 255	768
gaa agc ttc aac ggc cag gtt ccg aga gat gac gaa cct gct ccg caa Glu Ser Phe Asn Gly Gln Val Pro Arg Asp Asp Glu Pro Ala Pro Gln 260 265 270	816
tct tct agc agc gct ccg gca tct tct agc agt gtt ccg gca agc tcc Ser Ser Ser Ala Pro Ala Ser Ser Ser Ser Val Pro Ala Ser Ser 275 280 285	864

tct agc gtc cct gcc tcc tcg	agc gca ttt gtt ccg ccg	agc tcc	912
Ser Ser Val Pro Ala Ser Ser Ser	Ala Phe Val Pro Pro Ser Ser		
290	295	300	
tcg agc gcc aca aac gca atc cac	gga atg cgc aca act ccg	gca gtt	960
Ser Ser Ala Thr Asn Ala Ile His	Gly Met Arg Thr Thr Pro Ala Val		
305	310	315	320
gca aag gaa cac cgc aat ctc gtg	aac gcc aag ggt gcc aag	gtg aac	1008
Ala Lys Glu His Arg Asn Leu Val	Asn Ala Lys Gly Ala Lys Val	Asn	
325	330	335	
ccg aat ggc cac aag cgt tat cgc	gtg aac ttt gaa cac taa		1050
Pro Asn Gly His Lys Arg Tyr Arg	Val Asn Phe Glu His		
340	345		

<210> 7  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Primer

<400> 7  
tcaccaccat ggtagcgca aag 23

<210> 8  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Primer

<400> 8  
gccacgaatt ctgttcaaag ttcac 25

<210> 9  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Primer

<400> 9  
cagccggcga tggccatgg tagcgca 27

<210> 10  
<211> 29  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Primer  
  
<400> 10  
ctgctagaag aattcggagc aggttcgtc 29  
  
<210> 11  
<211> 6  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: Synthetic  
illustrative peptide  
  
<220>  
<221> MOD\_RES  
<222> (2)  
<223> An uncharged residue, such as Alanine, Proline or  
Glutamine  
  
<400> 11  
Pro Xaa Ser Ser Ser Ser  
1 5